

UNITED STATES PATENT APPLICATION

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for

**METHODS AND APPARATUS TO IMPROVE THE SENSITIVITY AND REPRODUCIBILITY OF
BIOLUMINESCENT ANALYTICAL METHODS**

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Tina Thomas

Title Of Invention

**METHODS AND APPARATUS TO IMPROVE THE SENSITIVITY AND REPRODUCIBILITY OF
BIOLUMINESCENT ANALYTICAL METHODS**

Prior Application

[0001] I claim priority benefits under 35 U.S.C. §119(e) of U.S. Provisional Patent Application Serial No. 60/193,974 filed March 31, 2000.

Field Of The Invention

[0002] The invention relates to techniques and apparatus for detecting light produced by a chemical reaction and known as chemiluminescence. Particularly, the invention relates to a hand-held ATP-chemiluminescence detection device used primarily in the food processing and food preparation industries for assessing cleanliness of various surfaces.

Background Of The Invention

[0003] Sanitary concerns as well as federal and state regulations in food processing and food preparation industries necessitate a device capable of rapidly and efficiently detecting various test samples from materials or surfaces. Various test apparatuses and test methods have been developed for that purpose. For example, it is widely desirable to determine or to test through quantitative and qualitative tests food, such as meat products, fruit, vegetables, and to detect for alkaline phosphates, salmonella, drugs, and antibiotics, such as; for example, various bacteria and pathogenic combinations, either in materials or on the surface of materials, or both.

[0004] The present commercial tests for the detection of ATP-luciferase reaction are generally directed to a chemiluminescence test, which ordinarily employs premeasured and prepackaged separate test reagents

mixing with the test sample to produce chemiluminescence. Accordingly, the count corresponding to the concentration of the ATP, which is determined by measuring or counting of the chemiluminescence, is compared against certain accepted control standards, or a threshold of a control standard.

[0005] Photomultiplier consumable-based detectors have been typically used to monitor the ATP-luciferase reaction. A photomultiplier consumable or PMT, capable of responding on an individual photon-by-photon basis amplifies low-level light intensity generated by chemiluminescence. As any light amplifying device, PMT is a delicate device that is not particularly suited for use by an unsophisticated user typically employed in food processing and food preparation industries and is bulky and costly requiring employment of a complicated manufacturing process.

[0006] Another device capable of generating an output current in response to low-level light signals is a photodiode, which is known to generate currents substantially lower than PMT. To convert currents from PMT into a useful electrical representation, an analog front-end circuit, such as the transimpedance amplifier, has been employed in devices used to measure chemiluminescence. As shown in FIG. 1, a typical transimpedance amplifier uses resistance to provide a real time linear representation of the light source. Input current generated by a photo-detector flows through the feedback resistor, R_F , to create a proportional output voltage $V_O = -I_{IN}R_F$. Accordingly, since R_F determines the transimpedance gain (amplification), very large values (gigaohm) of the feedback resistance R_F are required to measure small signal input current. However, transimpedance amplifiers with such high values of the feedback resistor are notorious for production problems – since the resistor and circuit board must be extremely clean to prevent stray feedback paths that otherwise will lower the gain of the amplifier, which can be detrimental for devices used to detect low level signals generated as a result of chemiluminescence. Also, to maintain the desirable cleanliness is

even more difficult in the food production and food processing industries where such devices are employed.

[0007] Also, the dynamic range of the transimpedance amplifier is limited unless gain switching is employed. Such gain switching includes a plurality of feedback resistors having different values. The higher the resistance value is, the higher the gain of the amplifier. The low photocurrent levels prevent the use of solid-state relays. Therefore, gain switching requires reed relays, which would make a device employing this amplifier less rugged because any mechanical structure is easily worn out.

[0008] To reduce the wear of the relays, the transimpedance amplifier makes the measurement at the "typical" gain level. This approach increases the time spent on the measurement, which is undesirable because the specifics of the food processing industry require that tests be performed frequently and in great number.

[0009] Furthermore, the transimpedance amplifier and reed relays require separate supply voltages necessitating separate dc-to-dc converters for battery operation, which leads to increased dimensions of a testing device utilizing the transimpedance amplifier. Discrete samplings used in the testing device means momentary high light levels because of the direct exposure to room light. As a consequence, the charge is collected at a capacitor which is necessary to be discharged before the next test is conducted. Thus, a decay rate for the impedance amplifier corresponds to the fixed RC time constant making the user wait before a subsequent test can be performed.

[00010] The high front-end amplification typically makes a measuring system sensitive to environmental changes, particularly temperature drift. Typically, acquiring a baseline signal immediately before the desired signal, and subtracting the baseline signal measurement from the signal measurement correct such baseline shifts. However, this technique brings

reliable results only if the baseline change is slowly varying, which is not the case with the food processing and food preparation industries where for example a cold storage room may be located next to a kitchen thus providing substantial temperature drift.

[00011] Still another measurement technique based on the photodiode detection includes a baseline measurement immediately before and after the desired signal measurement, and an average baseline signal is then subtracted from the signal measurement. Similarly to the above-discussed method, this correction can work well as long as the change in the baseline with time is nearly constant.

[00012] Finally, by monitoring the baseline signal it is sometimes possible to account for a non-uniform change in the baseline by fitting the change in the baseline to a known response function, and subtracting the calculated baseline from the measured signal.

[00013] Such procedures can only work if there is a known time period in which to acquire one or more baseline readings.

[00014] The technique of measuring the baseline before/after the sample measurement period requires the use of a shutter or some other means of ensuring that a sample is not resulting in a photodiode current, which has been associated with a few problems in case of a hand-held device. The currents generated when the shutter moved detrimentally affected the final measurement. Furthermore, both the shutter and a motor for actuating the shutter were too big to fit in the desired package. Also, the power requirements of the motor would have added substantially to the power drain placed on the batteries. Finally, the use of shutter assembly contributed to the relatively high cost and the poorer reliability.

[00015] It is known that many photodetecting transducers used for the detection of luminescence are very sensitive to static charge; for instance, static charges seen when a sample consumable is inserted into the sample compartment. Conventionally, a sample compartment of known devices must be made of a conductive material or some other means must be provided to drain static charge from the sample consumable. In most cases, it is difficult to achieve the required intimate contact between the sample compartment and a conductive wall of the sample compartment to quickly drain the static charge. In other cases, it is difficult to use a conductive sample compartment, which makes it very difficult to ensure that any static charge retained on the sample consumable does not influence the signal output.

[00016] Also, a shutter is used to shield a photodiode from direct exposure to high light levels which can damage the detection circuitry and often will increase noise. Unfortunately, any static charge or potential difference on a shutter mechanism will result in a transient signal as the shutter is moved across the photodiode. Given the low-level of the photogenerated currents that must be detected, nonconductive or partially conducting surface films on the shutter can cause associated capacitances and potential differences that result in large transients that may be much larger than the photogenerated currents.

[00017] Finally, particularly in hand-held instruments of the described-above type it is difficult to fully shield the high sensitivity detection system completely to reduce spurious noise effects. This is a particular acute problem along the optical path, since most optically transparent material are nonconductors.

[00018] It is, therefore, desirable to have a hand-held device provided with a photodiode-based detection system for monitoring the ATP-luciferase reaction to provide a cost-efficient hand-held device. Furthermore, it is desirable to provide the hand-held device wherein the photodiode-based

detection system for monitoring the ATP-luciferase reaction is coupled with a switched integrator to overcome the drawbacks associated with the transimpedance amplifier. Also desirable is the hand-held device wherein negative effects of static charge on a photodiode-based detection system are minimized.

Summary Of The Invention

[00019] In accordance with the invention, a hand-held assay device has a photodiode transducer, which is capable of generating an output current in response to low-level light signals generated by the ATP-luciferase reaction, and a switched integrator, which integrates a sample signal corresponding to the low-level input current for a user-determined period.

[00020] To increase the precision of detection and to automatically compensate for environmental changes, such as temperature and humidity, the inventive assay device further utilizes two channels of an available switched integrator to simultaneously measure both a reference signal indicative of the environmental changes and the sample signals. Thus, in addition to the sample signal generated in response the chemiluminescence by the sample photodiode, a reference photodiode, blocked from light detects the dark current due to the environmental changes and generates the reference signal. Since the photodiodes are in close proximity, the effects of these environmental changes are canceled providing excellent baseline correction even when the system is subjected to rapid temperature swings. Any correlated noise on the photodiodes will also cancel out.

[00021] In accordance with another aspect of the invention, the assay device can provide a self-calibrating test, wherein a LED generates a signal on demand, which is measured by the first photodiode mounted immediately below transparent window, indicating the desired cleanliness of the window.

[00022] According to still another aspect of the invention, the assay device has electronics allowing an automatically determine integration time period to be used so that a measurement is made before the integrators reach the state of saturation.

[00023] It is therefore an object of the invention to provide a hand-held assay device that makes possible the rapid detection through chemiluminescence of the presence of ATP on a surface.

[00024] Still another object of the invention is to provide an assay device that is operated by unskilled operators under the relatively harsh field environment of institutional food service preparation services, health care providers and the like.

[00025] Still another object of the invention is to provide an assay device having a cost efficient detection system that includes at least one photodiode capable of detecting the chemiluminescence signal.

[00026] A further object of the invention is to provide an assay device having a system for amplifying low-level signals generated by a photodiode in response to the detection of the chemiluminescence and including at least one switched integrator.

[00027] Still another object of the invention is to provide an assay device capable of automatically compensating for changes in detector baseline, independent of environmental changes and effect these change have on a front-end switched integrator.

[00028] Yet another object of the invention is to provide an assay device capable of automatically evaluating the cleanness of its transparent parts before an analytical test has been conducted.

[00029] A further object of the invention is to provide an assay device in which noise generated by static charge is minimized.

Brief Description Of The Drawings

[00030] The above and other objects, features and advantages will become more readily apparent from the following specific description accompanied by the following drawings, in which:

[00031] FIG. 1 is a transimpedance amplifier used in the prior art hand-held devices for detecting chemiluminescence.

[00032] FIG. 2 is a perspective view of an assay device in accordance with invention shown with an inventive detection circuit.

[00033] FIG. 3 is a flow chart illustrating an operation of the device of FIG. 2.

[00034] FIG. 4 is a flow chart illustrating a calibration mode of the device shown in FIG. 2.

[00035] FIG. 5 is a flow chart illustrating a consumable-detection mode in accordance one embodiment of the device of FIG. 2.

[00036] FIG. 6 is a flow chart illustrating another embodiment of the consumable-detection mode of the device shown in FIG. 2.

[00037] FIG. 7 is a flow chart illustrating another embodiment of the consumable-detection mode of the device shown in FIG. 2.

[00038] FIG. 8 is a flow chart illustrating an analytical mode employed in the device shown in FIG. 2.

[00039] FIG. 9 is a flow chart of a manual mode of the device shown in FIG. 2.

[00040] FIG. 10 is a sectional view of a transparent window located along an optical path between a sample chamber and electronics of the device shown in FIG. 2.

[00041] FIGS. 11A-11C graphically illustrate a measuring technique employed in the device of FIG. 2.

Detailed Description Of The Drawings

[00042] Referring to FIG. 2, a hand-held assay device 10 includes a consumable 12 removably inserted in a sample compartment 13 and containing a sample which as a result of ATP-luciferase reaction can generate a low level chemiluminescence, as explained in a co-pending patent application No. which is owned by the same assignee and fully incorporated herein by reference.

[00043] In accordance with the invention, detection of this low-light level chemiluminescence is detected by a photodiode 14 placed in a housing of the device in the close proximity of the sample. The photodiode is sensitive to femtoamp-level currents (10^{-15} amps) detected by a system which is fronted by a switched integrating amplifier or switched integrator 18. The switched integrator reduces the analog circuitry, which is characteristic of the transimpedance amplifier, and brings digitization process closer to the photodiode placed across the inputs of the switched integrator.

[00044] As shown in FIG.2, the switched integrator 18 is used with a fairly low integration capacitor 22 (10-500pF) in a feedback loop. This is combined with a reset solid-state switch 26. The switch integrator operates by collecting the photodiode output charge on the integration capacitor 22

while the reset switch 26 is open. Once it is closed, the output of the switched amplifier equals to zero volts. When this is accomplished the reset switch is open again starting the process all over again. The analog gain of this input stage is changed with values of the integration capacitor 22, or preferably, with changing the integration time, defined as the time duration during which the reset switch is open.

[00045] The digitized signal is further sent to a controller, which is typically a microprocessor. If the integrated value of the signal is equal to or greater than the reference or threshold value after applying the necessary corrections, a display 36 indicates the presence of the microorganisms in a suitable form. If, however, the output signal has not quite reached a measurable value, the integration time during which the reset switch is open can be incrementally increased by software executing on the microprocessor, as will be explained below.

[00046] In comparison with the transimpedance amplifier, the switched integrator has an extended dynamic range of 10^8 and does not require mechanical relays for switched gains. Furthermore, the switched integrator does not require a separate +/- voltage supply for the operational amplifiers that would be used for a low-noise/high gain transimpedance amplifier.

[00047] With the single switched integrator 18 and the single photodiode, adequate detection requires that each measurement be integrated for up to 30 seconds. An extended measurement period renders a device equipped with the photodiode difficult to use, because the user is typically required to provide frequent tests in a short period of time. In addition, since the baseline is changing during the measurement period, the long measurement period makes it more difficult to ensure that the baseline measurement will correctly estimate the baseline level during the signal measurement, particularly since the observed change is not unidirectional.

[00048] To make the measurement of the chemiluminescence less sensitive to the environmental changes, a second or reference photodiode 16 is placed in close proximity of the first photodiode 14. However, this photodiode 16 is reliably shielded from any source of light including chemiluminescence light and generates an input current primarily corresponding to environmental changes. As long as baseline and signal photodiodes 16, 14, respectively, are closely positioned, any environmental changes, such as temperature and correlated noise, can be cancelled. Thus, $L_{res} = (L_s + T^0 + N_{corr}) - (L_{ref} + T^0 + N_{corr}) = L_s - L_{ref}$, wherein L_s is an integrated signal generated by the first photodiode 14 and integrated by the integrator 18, and L_{ref} is an integrated signal generated by the photodiode 16 and integrated by an integrator 20.

[00049] The embodiment including two photodiodes provides much better detection than a single photodiode. However, there are some applications where the best performance is not needed and thus the single photodiode structure can be successfully employed.

[00050] The concept illustrating the two-photodiode structure is shown in FIGS. 11A-11C, wherein FIG. 11A illustrates the signal from the photodiode 14 integrated by the switched integrator in response to the chemiluminescence. FIG. 11B illustrates the integrated signal generated in response to the temperature swing by the photodiode 16, and FIG. 11C shows the combined integrating resulting signal L_{res} . This signal accounts for the changes in the light signal caused by the signal corresponding to the temperature, humidity, mechanical impact and the like drift.

[00051] Structurally, the integrator 20 is identical to the previously disclosed integrator 18 and includes an integrator feedback capacitor 24, a reset switch 28 and a respective A/D converter 32. The measurement of the baseline and signal inputs are simultaneously processed during the same integration time and integrated outputs of the switched integrators are

combined in the controller 34, wherein the baseline output signal is subtracted from the signal output signal, as seen in FIGS. 2 and 3.

[00052] The overall operation of the device, as shown in FIG. 3, includes an initialization or calibration mode 44, wherein transparency and/or cleanliness of components forming a light path are determined. The device further has a consumable-detection mode, wherein the presence of the consumable 12, which is used to swipe the surface for collecting a sample to be tested on ATP or other entity, in the sample compartment is verified at 46. Finally, the device has an analytical or operation mode 48, wherein the sample is tested on ATP. Particularly, the sample and reference signals (Ls, Lref) corresponding to currents generated by photodiodes 14, 16, respectively, are integrated by integrators 18 and 20, digitized in 32 and inputted in the controller 34. The controller has software for subtracting the reference signal from the sample signal. If the resulting signal is at least equal to the threshold stored in a memory of the controller, the display 36 informs the operator about the presence of ATP. The integration period will be increased if the resulting signal is lower than the reference value in accordance with a flow chart shown in FIG. 8.

[00053] Alternatively, a subtraction circuit can be implemented before the signals are fed into the microprocessor. However, being able to read the individual digitized signals prior to the subtraction provides some signal processing advantages, such as an ability to make decisions based on individual signal/baseline levels.

[00054] Referring to FIGS. 1 and 4, the calibration mode is shown. To provide a reliable measurement of ATP, optical components, such as a transparent window 41 (FIGS. 1 and 10) that locates along the light path and separates the consumable 12 from the photodiode 14, have to be clean within the expected intensity of a source of light, such as an LED 40 mounted in the sample compartment. Detection of a signal emitted by the LED is analogous

to the technique employed for the ATP detection and is performed by a combination of photodiodes and switched integrators. The calibration mode begins with turning on the LED 40 generating a signal, which has the expected LED intensity range, at powering up the device. An integrated value of a resulting signal processed by the controller is compared with the reference value (L_{ref}) set and stored in the memory of the controller during a manufacturing stage at 52. If the cleanliness signal L is within the expected range of the LED intensity, the device is ready for further operations. A correction value indicative of the relative cleanliness of the window is stored for final adjustment of a measured ATP signal at 70 (FIG. 8). If the cleanliness signal, which appears on the display, indicates that the window is soiled beyond the range, the user is required to clean the window before the device will be ready to conduct the analytical test.

[00055] Furthermore, the analytical test for detecting ATP cannot be performed unless the presence of the consumable is verified, as shown in FIG. 5. Initially, the LED is on, as indicated by 54, and the signals from the photodiodes 14 and 16 are integrated, digitized and inputted in the microprocessor 34 where a resulting signal $L_1 = L_s - L_r$ is measured. If the resulting signal exceeds a level above the expected LED intensity range at 56, which is equal to NL_{led} , wherein L_{led} is the stored LED reference value, it means that this signal is emitted by a high-ATP sample which can only be contained in the consumable which has been inserted in the sample compartment, as shown at 66.

[00056] If mid-range light level is detected at 58, which is within the range of the expected LED intensity and similar to the stored value of the LED, the consumable might be present. To verify its presence, software executing on the microprocessor turns the LED off at 60, provides a measurement of a resulting consumable signal L_2 and, if this signal is substantially the same as L_1 , then the consumable is present, as shown at 66.

[00057] If low light level is detected with the LED on at 64, the consumable is blocking the beam. Finally, if the resulting signal $L_1 - L_2$ is below the low-level light intensity, then the consumable has not been inserted, as indicated at 68, and a signal that has been detected corresponds to a dark current in the reference photodiode 16.

[00058] Alternatively, as shown in FIG. 6, the consumable detection mode has the LED off at 72. If the resulting signal L_1 measured at 74 has the high or mid-light level, as shown at 76, the ATP is present at 84.

[00059] If the detected signal substantially corresponds to the low-light level shown at 78, which is consistent with the dark current generated by photodiode 16 dark, a sample might be present. To verify the presence of the consumable, software executing on the microprocessor turns on the LED at 80 to measure a resulting signal L_2 at 80. If the L_2 signal is similar to L_1 , as indicated at 81, then the consumable is present. If the low level has not been detected, there is no consumable in the sample compartment, as shown at 86.

[00060] A particularly simple embodiment, wherein the presence of the consumable is verified, is shown in FIG. 7 where software executing on the microprocessor first turns on the LED and measures a first L_1 at 88 in accordance with the technique explained above. Then the LED is turned off at 90 to provide a measurement of the second resulting signal L_2 , and if the $L = L_1 - L_2$ higher than an upper limit or lower than a lower limit, as indicated by 92, the consumable is present at 94. Otherwise, the analytical test will be aborted, as indicated by 96. The procedures disclosed in reference to FIGS. 5-7 may be continually performed before conducting the analytical test until the consumable is detected.

[00061] Alternatively to optical detection of the consumable, as disclosed above, it is possible to use a mechanical microswitch to provide the detection

mode. In this case, the microswitch is strategically mounted in the sample compartment to be in direct contact with the insertable consumable. Obviously, actuation of the microswitch indicates the presence of the consumable. However, advantage of optical detection is that a mechanical microswitch would be much easier to contaminate, and then more difficult to seal.

[00062] After the initialization and consumable detection modes have been completed, the device is ready to conduct the analytical test, as shown in FIG. 8. The analytical mode is directly dependent on how rapidly ambient parameters change. Under normally changing conditions, the analytical test first includes discharging photodiode charge accumulated on the feedback capacitors, which detrimentally affects a measurement of ATP. This is attained by software executing on the microprocessor which closes/opens integration switches (26, 28) at least once at 98, but if necessary more than once. Initially, a short integration time, for example 0.5msec, is set at 100. If the resulting signal $L_{res}=L_s-L_r$ is less than the predetermined threshold, as shown at 102, a larger integration time is set at 104. This sequence continues unless a satisfactory quantity of ATP is determined. However, an integration period is continuously controlled not to exceed a predetermined value at 106, for example 5 seconds. At this point the test is completed.

[00063] However, if the environmental changes are rapid, the switched integrator can reach a saturation mode substantially sooner than the 5-second limit. In that case, the shorter integration time result can be used to predict that integrator saturation could occur at next predetermined integration time. Note, the expected baseline is known. This is particularly true at short integration time because it is difficult to expect any change to achieve a critical level within, for example, a 0.5msec period. Then if the 0.5msec integration time signal is below baseline to the extent that 10 times that negative offset would be less than zero—than obviously, 5sec integration time measurement cannot be made. As a result, software executing on the

controller can dynamically change the integration time limit from 5 seconds to, for example, 2 seconds, as shown at 101.

[00064] The threshold is selected such that the sample-to-sample variation is dominated by the process of sampling and mixing instead of being defined by the signal-to-noise ratio of the electronics. In other words, the threshold is selected to be N times greater than the empirically determined inherent noise of the electronics. Specifically the minimum counts at any integration time corresponds to about a 1% of relative standard deviation (RSD), while sampling/mixing will cause measured precision to be more than 5%. Thus, dynamically changing the integration time in accordance with software executing on the microprocessor allows the device to operate with the best possible signal-to-noise ratio for the given environmental conditions including temperature, humidity, external shocks and the like.

[00065] After the ATP has been detected, the signal representing it is corrected at 70 in accordance with the correction value and a logarithmic number of the corrected signal value is calculated and displayed.

[00066] Note, none of the modes can be performed unless a door 38 (FIG.1) allowing access to the sample compartment 13 is closed. Software executing on the microprocessor is able to verify the closed state of the door. Furthermore, the device will not operate even if a small hole is formed in the lid. Obviously, during the first detection of light coming through the hole, the device will read a signal as the one emitted by a high-ATP sample. However, during the next measurement indicating the high-ATP sample, the device will warn a user to check if the lid is damaged.

[00067] FIG. 9 illustrates a manual mode of the device, wherein a user, based on local requirements, is able to set a new limit for indicating the presence of ATP. Typically, if the measured quantity of ATP exceeds the threshold number stored in the memory of the controller and assumed to be

indicative of the ATP, the measured number appears on the display. However, local requirements that can be different from a criteria used during the manufacturing stage may necessitate a user to choose a different threshold number, which will be indicative of the ATP. To do so, the microprocessor turns on the LED at 110 in response to powering up the device. After verifying the absence of the consumable at 112, the user using the menu can set a desirable number at 106. If a given measurement meets the newly set number, a check mark indicative of the sufficient quantity of ATP along with the number appear on the screen.

[00068] Similarly to the environmental changes, static charges, such as those seen when the sample consumable is introduced in the compartment, negatively affect the inventive detection system. The highly current-sensitive photodiode 14 of the present invention is also sensitive to spurious static charges. Also, the inventive structure makes it very difficult to use a metal sample compartment, semi-conductive plastics, although available, still have a surface resistance of about 10^6 ohms/sq making it likely that this resistance will not eliminate signal transients due to the static charge on the sample consumable.

[00069] To minimize the effect of these charges, the window 41, as shown in FIG. 10, is made of glass has its side, which faces the sample compartment, coated with a transparent conductive coating 116. The coating is in contact with an electro-conductive chassis 118 of the device to form and act as a Faraday cage. Preferably, this coating is indium tin-oxide (ITO). A bottom side of the window is coated with a layer 120 acting as a bandpass filter to limit the light striking the photodiode 14.

[00070] It is foreseen within the scope of this invention to place the ITO on the window of photodiode, since typically the case of photodiode is conductive, and it ensures a dissipation path of any transferred charge to the ground.

[00071] Also, it is possible to cover only the bottom side of the window with the ITO, whereas the opposite side is provided with a band pass or band limited filter. Note, the filter can be a coating or is a made of a whole body. In this case, a wavy washer needs to be interposed the photodiode 14 and the coating to provide electrical contact between these components.

[00072] Thus, discharge effects resulted from closing/opening of the integration switches and having the ITO coating eliminate the need in a mechanical shutter. This, in turn, generates significant cost savings while also increases the reliability of the instrument through the elimination of a complicated mechanical assembly, such as the shutter.

[00073] Although the reference photodiode effectively eliminates the effects the environmental changes, it has been found that an optic is still required between the sample photodiode and the consumable, because the consumable is often not at thermal equilibrium with environment. This necessitates a larger distance between the sample photodiode and the transparent window. On the other hang, the increased distance affects an optical path. Clearly, the closer the source of light to the transparent window and, therefore, to the photodiode, the less distortion of the light beam. This dichotomy can be reconciled by introducing a combination of two plano-convex lens 122, 124 between the consumable 12 and the transparent window 41.

[00074] Although the invention has been described with reference to a particular arrangements of parts, features and the like, these are not intended to exhaust all possible arrangements or features, and indeed many other modifications and variations will be ascertainable to those of skill in the art.